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14. ABSTRACT

Breast cancer incidence and outcomes (disease-free survival and overall survival) vary widely in women of different racial and ethnic backgrounds. Clinical research indicates that many possible factors, including ethnicity and tumor biology, affect outcomes in women diagnosed with breast cancer. Regional lymph node status (presence of metastasis in regional lymph nodes and number of affected nodes) is the best prognostic indicator for women with breast cancer. It is not known if ethnicity affects lymph node status or if the value of promising prognostic indicators under study, such as low molecular weight Cyclin E, is independent of ethnicity. In this study we show that the LMW isoforms of cyclin E are more highly expressed in African American and Hispanic patients compared to White patients. Furthermore, Hispanic patients with a positive sentinel lymph node have a worse prognosis than other subgroups. LMW cyclin E is also associated with the aggressive, triple negative form of breast cancer that also results in decreased survival. Therefore, LMW cyclin E is associated with different subgroups of breast cancer patients that have poor survival, and could provide a future therapeutic target for a subset of breast cancer patients.

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Breast Cancer, Cyclin E, ethnicity

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Introduction:

Breast cancer incidence and outcomes (disease-free survival and overall survival) vary widely in women of different racial and ethnic backgrounds. Clinical research indicates that many possible factors, including ethnicity and tumor biology, affect outcomes in women diagnosed with breast cancer. It is not known if ethnicity affects lymph node status or if the value of promising prognostic indicators under study, such as low molecular weight Cyclin E, is independent of ethnicity. The objective of this study is to further describe the differences in disease-free survival and overall survival by ethnicity for women diagnosed with breast cancer.

Body:

Specific Aim 1- To evaluate the relationship between ethnicity and sentinel lymph node (SLN) status in women diagnosed with breast cancer we collected clinical data on 251 breast cancer patients that were treated at The University of Texas- M.D. Anderson Cancer Center (MDACC) and underwent surgical treatment for removal of the primary tumor. As a component of their surgical management, subjects underwent sentinel lymph node biopsy (SLNB) with or without completion axillary lymph node dissection (ALND). Clinical data including hormone receptor status, stage of disease and recurrence events were recorded for each patient. The median follow up for this patient cohort was 4.27 years. The ethnic classification of the cohort was: White, n=190; Black, n=13; Hispanic, n=22 and Asian, n= 11. We performed a univariate analysis for disease free survival (DFS), disease specific survival (DSS) and overall survival (OS) (Table 1).

p value for univariate analysis

variable name	DFS	DSS	OS
Race_Hispanic & SLN status	0.004	0.0009	0.052
SLN status	0.0013	0.0028	0.057
race_Hispanic	0.676	0.104	0.431
age ≥ 55	NS	NS	NS
Other Malignancy	NS	NS	NS
Other Breast Cancer	0.072	NS	NS
Side	NS	NS	NS
Bilateral	0.022	NS	NS
LVI	0.0002	0.0062	0.00016
ER status	<0.0001	<0.0001	0.015
PR status	NS	NS	NS
Her-2/neu status	NS	NS	NS
T Stage	<0.0001	<0.0001	<0.0001
Pathstage	<0.0001	<0.0001	<0.0001
Recurrence	<0.0001	<0.0001	<0.0001
local recurrence	<0.0001	NS	NS
regional recurrence	<0.0001	<0.0001	<0.0001
distant recurrence	<0.0001	<0.0001	<0.0001
Surgery	0.0064	0.018	0.0054
radiation treatment	0.04	NS	NS
Neoadjuvant hormonal tx	NS	NS	NS
Neoadjunvant chemo tx	0.0741	0.0011	0.04
Adjunvant hormonal tx	NS	NS	NS
Adjuvant chemo tx	0.0002	0.021	0.055

In summary, expected results were observed such as the significant relationship between stage and survival as well as presence of lymphovascular invasion (LVI) and survival. Those patients with increased stage of disease or LVI had a significantly reduced survival. Our data also shows that among patients who had a positive SLN, Hispanic patients had worse survival outcomes (DFS, DSS and OS) than other ethnicities. Since there was only one Black patient in this group, we can't estimate the survival for this race. Other significant prognostic factors from univariate analysis include SLN status, LVI, ER, T_Stage, Pathstage,

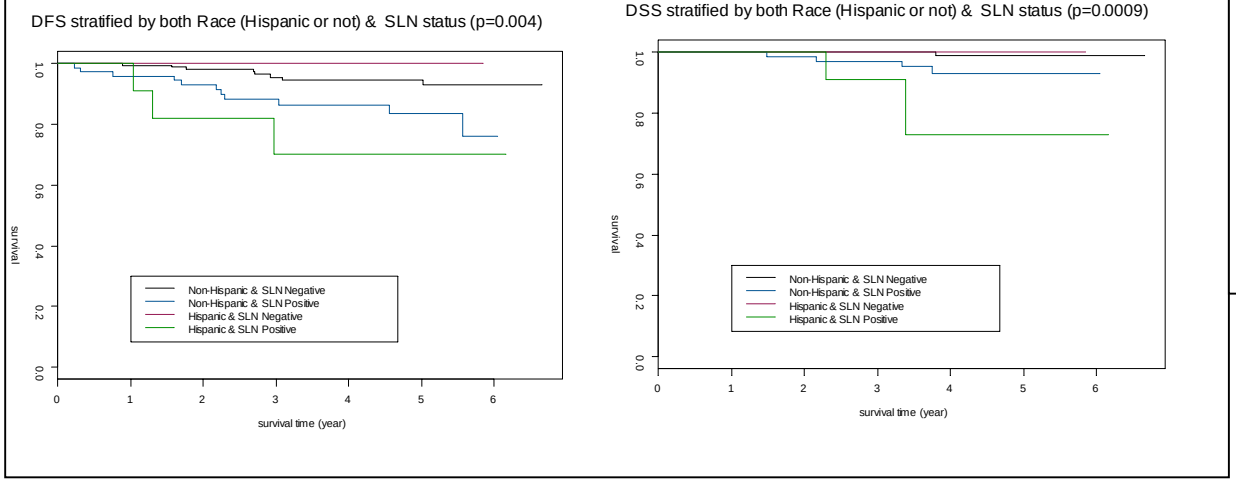

Figure 1: Hispanic patients with positive sentinel lymph node have decreased survival. Kaplan-Meier curves were generated to compare disease-free (A) and disease-specific (B) survival in non-Hispanic and Hispanic patients with and without SLN positive status.

Recurrence, Surgery, XRT, Neoadjuvant chemotherapy treatment, and Adjuvant chemotherapy treatment. Figure 1 shows Kaplan-Meier curves demonstrating the significantly decreased DFS (Figure 1A) and DSS (Figure 1B) associated with Hispanic patients having a positive SLN status (the data highlighted in Table 1).

Specific Aim 2- The low molecular weight (LMW) isoforms of cyclin E have been shown to correlate with poor outcome for breast cancer patients(Keyomarsi et al., 2002) and have been implicated in the process of breast tumorigenesis(Akli et al., 2007; Wingate et al., 2003). Because breast cancer incidence and outcome varies in women of different racial/ethnic backgrounds, we aimed to determine the correlation between LMW cyclin E expression and the different ethnic groups. To evaluate the relationship between ethnicity and high levels of LMW cyclin E in women diagnosed with breast cancer, tumor tissue and normal tissue were removed from the patients (the same cohort as in specific aim 1) and protein lysates were used to perform western blotting. Cyclin E levels were determined by densitometry of the western blots, which allows for the quantification of the full-length cyclin E (FL) and the low molecular weight isoforms (LMW) of cyclin E. Figure 2 shows a bar graph of the mean densitometric values of FL cyclin E in the tumor along with the LMW cyclin E in both the normal and tumor tissues. We observed a significant difference in expression of LMW cyclin E in the Black and Hispanic patients compared to the expression in the white patients. As expected, there was very little LMW cyclin E in the normal tissue samples, and there was no difference in the expression of full-length cyclin E between the groups. We are continuing to process samples and follow-up on the patients in order to further correlate LMW cyclin E expression in different ethnicities with outcome. With more data we will be able to perfom multivariate analyses to determine whether LMW cyclin E expression is an independent prognostic factor for different ethnic subgroups. If, after further analysis, LMW cyclin E expression remains significantly different between ethnic populations, it will provide evidence that the tumor phenotypes are unique and require different treatment strategies.

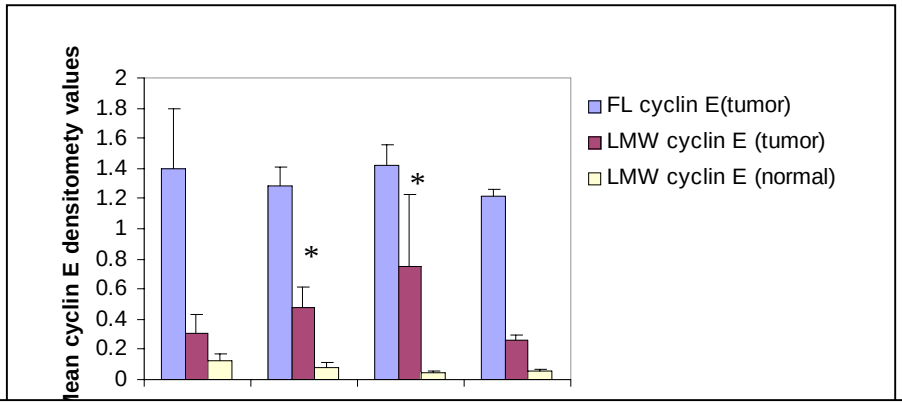

Figure 2: LMW cyclin E is expressed differentially between ethnic groups. Cyclin E western blots of normal and tumor tissue were subjected to densitometry. Values were normalized by dividing by the value for expression of full-length cyclin E in the normal tissue sample. *= p<0.05 by t-test compared to the white patients. Standard error is represented by the lines on top of each bar.

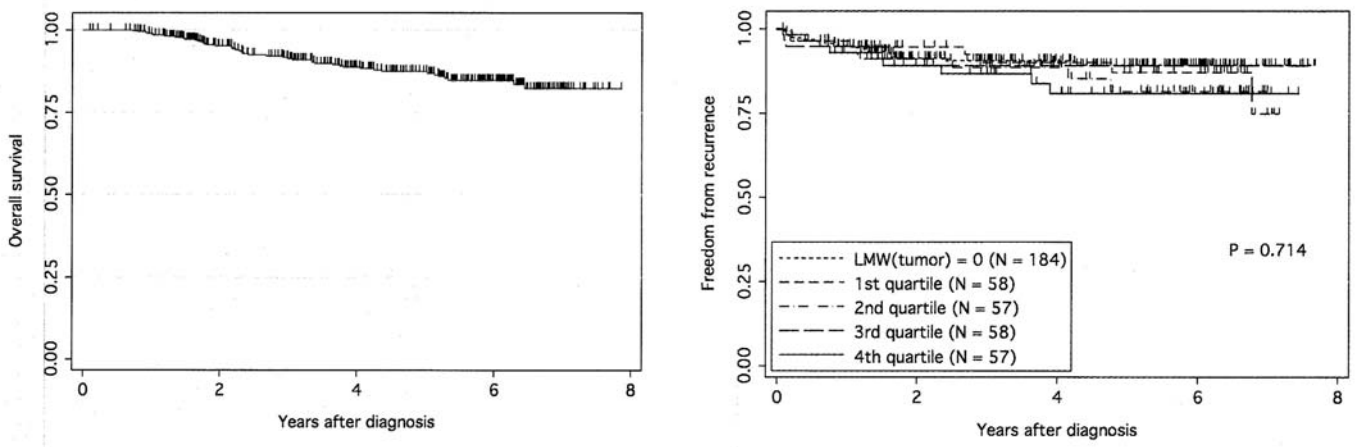

Figure 3: Longer follow-up and more patients are needed to determine whether LMW cyclin E correlates with survival of breast cancer patients. (A) Survival of 414 breast cancer patients enrolled in Lab00-222. (B) Patients are divided in to quartiles based on LMW cyclin E expression and followed for recurrence.

Specific Aim 3- To determine the prognostic and/or predictive value of LMW cyclin E we will need to continue to assess the cyclin E expression in normal and tissue samples from breast cancer patients. Although we can only report on a portion here, we are continuing to enroll patients on the protocol (IRB#LAB00-222) and to date 546 patients have been enrolled. We have found that the patients are experiencing a generally good survival (figure 3A). Out of the 415 patients studied, death from disease was reported for only 7 patients. Therefore, we looked at time to recurrence as an endpoint to determine whether there were differences in disease progression for patients with high versus low or no expression of LMW cyclin E. However, when patients were separated into quartiles depending on the level of LMW cyclin E expression in the tumor sample there was no significant difference in freedom from recurrence (figure 3B). Therefore, no conclusions can currently be drawn concerning the prognostic value of LMW cyclin E, or how it compares to the prognostic strength of lymph node status. Longer follow-up along with more patient accrual is underway to increase the recurrence and death events needed to generate statistically significant results.

A major concern in breast cancer research and treatment is the aggressive disease seen in patients with the subtype of breast cancer known as triple negative. Triple negative breast cancers are identified by the lack of ER, PR and Her2/Neu expression and therefore the lack of targeted therapy (i.e. hormonal or trastuzumab treatments). During our analysis of LMW cyclin E expression a striking correlation was observed between the expression of LMW cyclin E expression and triple negative status of breast cancer patients (Figure 4). LMW cyclin E expression is signinificantly higher in triple negative breast cancer

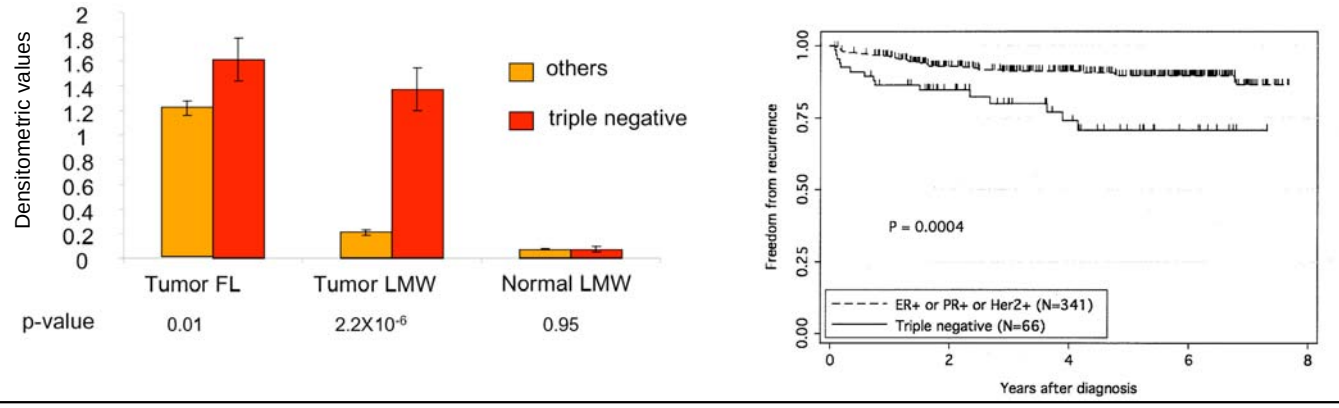

Figure 4: LMW cyclin E expression is significantly associated with a subset of breast cancer patient with poor survival. (A) Densitometry performed on western blots of cyclin E in tumor and normal samples. (B) Freedom from recurrence over time for patients stratified by receptor status of their tumors (triple negative or expression of ER, PR or Her2/neu)

patients compared to all other subtypes (those with expression of at least one of the receptors: ER, PR or Her2/Neu). There were no differences in the full-length cyclin E in the triple negative patients compared to others. Again, there was little LMW cyclin E in the normal samples (Figure 4A). We analyzed the freedom from recurrence in the triple negative patients compared to those with expression of one of the receptors and found that those with triple negative breast cancer had significantly reduced freedom from recurrence ($p<0.0004$, Figure 4B). For example, at 6 years post diagnosis, only about 75% of triple negative patients were recurrence free compared to about 90% of patients with receptor positive disease. Our other laboratory studies also provide evidence that overexpression of LMW cyclin E in cell model systems leads to a basal-like phenotype, similar to what is predominantly observed in triple negative patients. Because LMW cyclin E is tumor specific and significantly associated with a subtype of breast cancer that lacks specific treatments, LMW cyclin E may serve as a future therapeutic target.

Key Research Accomplishments:

This study is the first to prospectively examine the expression LMW cyclin E in different subgroups of breast cancer patients. In doing so, we have drawn the following conclusions:

(1) The low molecular weight (LMW) isoforms of cyclin E are expressed at higher levels in Hispanic and Black patients compared with Caucasian breast cancer patients.
(2) Hispanic breast cancer patients with a positive sentinel lymph node have significantly decreased survival compared to non-Hispanic patients, or Hispanic patients who do not have a positive sentinel lymph node.
(3) No conclusion can be drawn about the correlation of LMW cyclin E expression with survival or recurrence of breast cancer patients as we are continuing to accrue patients to our study and follow them for events.
(4) LMW cyclin E expression is significantly correlated with the triple negative subtype of breast cancer patients.

In conclusion, the LMW isoforms of cyclin E are associated with subtypes of breast cancers that are known to have poor outcome and lack specific treatments. Our studies, along with other ongoing studies in the laboratory, suggest that the LMW isoforms of cyclin E play a role in the biology of aggressive breast cancers and may provide a novel therapeutic target.

Reportable Outcomes

-M. A. Hassett, K. K. Hunt, K. Keyomarsi ; OUTCOMES BY ETHNICITY. SENTINEL LYMPH NODE STATUS IN WOMEN WITH BREAST CANCER
Era of Hope Department of Defense Breast Cancer Research Program Meeting Proceedings, June, 2005

-K. Keyomarsi, M.A. Hassett, A. Sahin, K.K. Hunt; CYCLIN E, A POWERFUL PREDICTOR OF SURVIVAL IN BREAST CANCER- A PROSPECTIVE STUDY
Era of Hope Department of Defense Breast Cancer Research Program Meeting Proceedings, June, 2005

-A manuscript will be submitted for publication when project is completed

- Study-specific database for unique study data will be generally available to other researchers as authorized by UTMDACC

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